



## PATENT ABSTRACTS OF JAPAN

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(22) Date of filing: 25.06.1992

(71) Applicant: MITSUI NORIN KK

(72) Inventor: HARA MASAHICO  
HONDA YOSHIKAZU

## (54) HYALURONIDASE ACTIVITY INHIBITOR

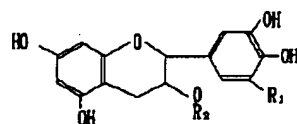
## (57) Abstract:

**PURPOSE:** To obtain a hyaluronidase activity inhibitor containing tea polyphenols such as epigallocatechin gallate as active ingredients, capable of inhibiting hyaluronidase activity having inflammatory action and useful as an anti-inflammatory agent, antiallergic agent, etc.

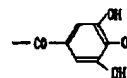
**CONSTITUTION:** The hyaluronidase activity inhibitor useful for medicine, etc., for reducing various kinds of inflammations and allergies is obtained by using one or more kinds of tea catechins of formula I ( $R_1$  is H or OH;  $R_2$  is H or group of formula II) such as epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin or (+)catechin and tea theaflavin of formula II ( $R_3$  and  $R_4$  are H, group of formula III) such as an isolated type theaflavin, theaflavin monogallate A, theaflavin monogallate B or theaflavin digallate as tea phenols extracted from tea as active ingredients and preparing these tea polyphenols alone or in combination with vehicles such as gelatin, a solvent such as

water or alcohol, diluent, etc., such as carboxymethyl cellulose.

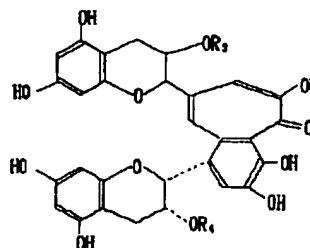
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I



II



III

TF -  $\alpha$  inflammation

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(11) **Publication No.** JP,6-9391,A  
(43) **Date of Publication** January 18, Heisei 6 (1994)  
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ABF      9360-4C  
7/00      D 9164-4C  
W 9164-4C  
7/48      9051-4C  
C07D311/62      7252-4C

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(71) **Applicant**

**Identification Number** 591039137

**Name** Mitsui agriculture-and-forestry incorporated company

**Address** 3-1-20, Nihombashi Muro-machi, Chuo-ku, Tokyo

(72) **Inventor(s)**

**Name** Hara Masahiko

**Address** 2-2-7, Minami-Surugadai, Fujieda-shi, Shizuoka-ken

(72) **Inventor(s)**

**Name** Honda Miwa

**Address** 2-7-23, Seko, Fujieda-shi, Shizuoka-ken

(74) **Attorney**

**Patent Attorney**

**Name** Kubota Fujiro

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(57) **Abstract**

**Elements of the Invention** The hyaluronidase activity inhibitor which contains tea polyphenol as an active principle.

**Effect** The hyaluronidase activity inhibitor of this invention does not have the worries about a side effect of as opposed to the body as drugs in order to use as a principal component the natural product extracted from the tea by which considerable-amount drink is carried out every day. And the hyaluronidase activity inhibitor of this invention checks activation of hyaluronidase remarkably by low-concentration addition. Therefore, the hyaluronidase activity inhibitor of this invention is used in order to mitigate various inflammation and

allergy as physisic, and also it can add for cosmetics etc. and it can prevent disassembly of hyaluronic acid.

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### **Claim(s)**

**Claim 1** The hyaluronidase activity inhibitor which contains tea polyphenol as an active principle.

**Claim 2** The hyaluronidase activity inhibitor according to claim 1 whose tea polyphenol is a least one sort of matter chosen from epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, (+) catechin, isolation mold theaflavin, and theaflavin monogallate A, theaflavin mono-gallate B, and theaflavin digallate.

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### **Detailed Description of the Invention**

#### **0001**

**Industrial Application** About a hyaluronidase activity inhibitor, this invention acts specifically **it is detailed and** to hyaluronidase, and relates to the hyaluronidase activity inhibitor which contains the tea polyphenol which checks the activation as an active principle.

#### **0002**

**Description of the Prior Art** Hyaluronidase is an enzyme which exists in the testis of an animal, snake venom, bacteria, etc., and is the hydrolase of the hyaluronic acid widely distributed over the connective tissue of an animal. It is known that hyaluronidase has the operation as a pathogenic drug. On the other hand, since activity is checked with an anti-inflammatory agent or an antiallergic agent, it is thought possible by checking hyaluronidase activity to mitigate inflammation and allergy. Furthermore, preventing disassembly of the hyaluronic acid mixed by cosmetics, such as a cream, a milky lotion, a lip stick, and a hair product, is also expected. Then, this invention persons tried development of the enzyme inhibitor which checks activation of hyaluronidase and does not have a harmful side effect to the body.

#### **0003**

**Means for Solving the Problem** this invention persons reached **that this matter is contained in tea and tea polyphenol, and** a header and this invention, as a result of repeating research wholeheartedly that not a chemical composition but the matter which has the drug effect made into the purpose out of a natural product should be searched. That is, this invention offers the hyaluronidase activity inhibitor which contains tea polyphenol as an active principle. The tea polyphenol which is the principal component of the hyaluronidase activity inhibitor of this invention is tea theaflavin expressed with the tea catechins expressed with the following general formula I, and a general formula II.

#### **0004**

#### **Formula 1**

ID=000002

**0005** (R1 shows H or OH among a formula, and R2 is H or **0006**.)

**Formula 2**

ID=000003

**0007** ) \*\*\*\*\*.

**0008** The following can be mentioned as an example of tea catechins expressed with the above-mentioned general formula I.

(-) Epicatechin (the inside of a general formula I, thing of R1 =H and R2 =H)

(-) Epigallocatechin (the inside of a general formula I, thing of R1 =OH and R2 =H)

(-) Epicatechin gallate (inside of a general formula I , and R1 =H, R2 = **0009**)

**Formula 3**

ID=000004

**0010** \*\*

(-) Epigallocatechin gallate (the inside of a general formula I, R1 =OH, R2 = **0011**)

**Formula 4**

ID=000005

**0012** \*\*

General formula II **0013**

**Formula 5**

ID=000006

**0014** (The inside of a formula, R3, and R4 are H or **0015**.)

**Formula 6**

ID=000007

**0016** An example and R3 And R4 Even if the same, you may differ.

**0017** Next, when the tea theaflavin expressed with the above-mentioned general formula I is shown concretely, there are the following.

Isolation mold theaflavin (the inside of a general formula II, thing of R3 =H and R4 =H)

Theaflavin mono-gallate A (inside of a general formula II , and R3 = **0018**)

**Formula 7**

ID=000008

**0019** The thing of R4 =H

Theaflavin mono-gallate B (inside of a general formula II , and R3 =H, R4 = **0020**)

**Formula 8**

ID=000009

**0021 \*\***

Theaflavin digallate (the inside of a general formula II, R3, R4 = **0022**)

**Formula 9**

ID=000010

## 0023 \*\*

The above-mentioned tea polyphenol can manufacture tea leaves as a raw material, and the process is indicated by JP,59-219384,A, the 60-13780 official report, the 61-130285 official report, etc.

**0024** It is used independently, and also it mixes with a suitable excipient, for example, gelatin, sodium alginate, etc., or the hyaluronidase activity inhibitor of this invention is used combining diluents, such as solvents, such as water and alcohols, and a carboxymethyl cellulose, etc. About the amount of the hyaluronidase activity inhibitor used of this invention, when medicating the body as drugs, the daily dose should just usually take 0.5-10g in taking orally so that it may become about 1-3g preferably. Dosage forms are arbitrary and are used as powder, a tablet, a capsule, etc. Moreover, when blending with cosmetics etc., the last concentration is 1-100 ppm. What is necessary is just to add so that it may become.

## 0025

**Example** Next, an example explains this invention in detail. In addition, measurement of inhibition ability was performed by the following approach based on the conventional method. Hyaluronidase activity inhibitor solution (1 mM) 50microl Enzyme (from Bovine testis and SIGMA shrine make) solution 100 mul (2,000 unit/ml buffer solution) is added and it is left for 20 minutes at 37 degrees C. next, enzyme activator (trade name: compound 48/80 and SIGMA shrine make) solution (0.1mg /ml buffer solution) 100microl is added -- after leaving it for 20 minutes at 37 degree C, Hyaluronic acid (from rooster comb and WAKO shrine make) solution (0.8 mg/ml buffer solution) 250microl which is a substrate is put in and was left for 40 minutes at 37 degrees C. 0.4N NaOH 100microl Morgan-Elson after stopping a reaction in addition -- strange method (Davidson, E.A., Aronson, N.N.:J.Bio Chem.242, 437 (1967)) of law The quantum of a product is performed. It asked for hyaluronidase activity inhibition ability. Inhibition activity was expressed with the rate of inhibition called for from the following formula. moreover the buffer solution -- 0.1 M The acetic-acid buffer solution (pH 4.0) was used. **0026**

**Equation 1** Rate (%) of inhibition =  $(A-B)-(C-D)/(A-B) \times 100$   
**0027** A: The absorbance B in 585nm of a reference solution : reference solution blank The absorbance D in 585nm of the absorbance C:inhibitor solution in 585nm: Inhibitor solution blank Absorbance in 585nm

**0028** The various tea polyphenol shown in the 1st table as an example 1 hyaluronidase activity inhibitor was used, and it asked for the hyaluronidase activity inhibition ability of each matter by the above-mentioned approach. A result is shown in the 1st table.

## 0029

### Table 1

**\*\* 1 Table** ----- An inhibitor The rate of inhibition (%)

----- (+) Catechin 26.8 Epicatechin 22.1 Epigallocatechin 17.6 Epicatechin gallate 55.5 Epigallocatechin gallate 40.3 isolation mold theaflavin 12.2 Theaflavin mono-gallate A 85.0 Theaflavin mono-gallate B 86.2 Theaflavin digallate

99.1----- **0030** In catechins and theaflavin, it was checked that epicatechin gallate, epigallocatechin gallate and theaflavin mono-gallate A, theaflavin mono-gallate B, and theaflavin digallate have strong hyaluronidase activity inhibition ability so that clearly from a table.

## 0031

**Effect of the Invention** The hyaluronidase activity inhibitor of this invention does not have the worries about a side effect of as opposed to the body as drugs in order to use as a principal component the natural product extracted from the tea by which considerable-

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(19)日本国特許庁(JP)

(12) 公 開 特 許 公 報 (A)

(11)特許出願公開番号

特開平6-9391

(43)公開日 平成6年(1994)1月18日

(51)Int.Cl. <sup>5</sup>	識別記号	庁内整理番号	F I	技術表示箇所
A 6 1 K 31/35	A E D	9360-4C		
	A B E	9360-4C		
	A B F	9360-4C		
7/00	D	9164-4C		
	W	9164-4C		

審査請求 未請求 請求項の数2(全 4 頁) 最終頁に続く

(21)出願番号	特願平4-190203	(71)出願人	591039137 三井農林株式会社 東京都中央区日本橋室町3丁目1番20号
(22)出願日	平成4年(1992)6月25日	(72)発明者	原 征彦 静岡県藤枝市南駿河台2-2-7
		(72)発明者	本田 美和 静岡県藤枝市瀬古2-7-23
		(74)代理人	弁理士 久保田 藤郎

(54)【発明の名称】 ヒアルロニダーゼ活性阻害剤

(57)【要約】

【構成】 茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤。

【効果】 本発明のヒアルロニダーゼ活性阻害剤は、日常相当量飲用されている茶から抽出される天然物を主成分とするため、薬剤としても人体に対する副作用の心配がない。しかも、本発明のヒアルロニダーゼ活性阻害剤は低濃度の添加でヒアルロニダーゼの活性化を著しく阻害する。したがって、本発明のヒアルロニダーゼ活性阻害剤は医薬として各種炎症やアレルギーを軽減するために用いる他、化粧品等に添加してヒアルロン酸の分解を防ぐことができる。

## 【特許請求の範囲】

【請求項1】 茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤。

【請求項2】 茶ポリフェノール類が、エピガロカテキンガレート、エピカテキンガレート、エピガロカテキン、エピカテキン、(+)カテキン、遊離型テアフラビン、テアフラビンモノガレートA、テアフラビンモノガレートB及びテアフラビンジガレートの中から選ばれた少なくとも1種の物質である請求項1記載のヒアルロニダーゼ活性阻害剤。

## 【発明の詳細な説明】

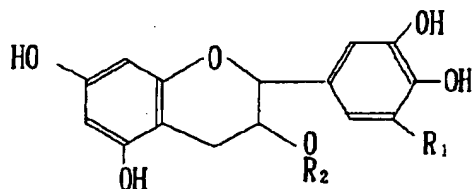
## 【0001】

【産業上の利用分野】本発明は、ヒアルロニダーゼ活性阻害剤に関し、詳しくはヒアルロニダーゼに特異的に作用して、その活性化を阻害する茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤に関する。

## 【0002】

【従来の技術および発明が解決しようとする課題】ヒアルロニダーゼは動物の睾丸や蛇毒、細菌等に存在する酵素であり、動物の結合組織に広く分布しているヒアルロ

ン酸の加水分解酵素である。ヒアルロニダーゼは起炎症\*

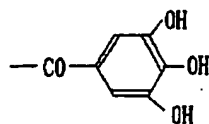


... I

【0005】(式中、R<sub>1</sub> はHまたはOHを示し、R<sub>2</sub> はHまたは

## 【0006】

## 【化2】



【0007】を示す。)

【0008】上記の一般式Iで表される茶カテキン類の具体例としては以下のものを挙げることができる。

(-)エピカテキン(一般式I中、R<sub>1</sub> = H, R<sub>2</sub> = Hのもの)

(-)エピガロカテキン(一般式I中、R<sub>1</sub> = OH, R<sub>2</sub> = Hのもの)

(-)エピカテキンガレート(一般式I中、R<sub>1</sub> = H, R<sub>2</sub> =

## 【0009】

\* 剤としての作用を持つことが知られている。一方、抗炎症剤や抗アレルギー剤により活性が阻害されることから、ヒアルロニダーゼ活性を阻害することにより、炎症やアレルギーを軽減することが可能であると考えられている。さらには、クリーム、乳液、口紅、ヘア製品などの化粧品に混合されたヒアルロン酸の分解を防ぐことも期待される。そこで、本発明者らはヒアルロニダーゼの活性化を阻害し、かつ人体に対して有害な副作用を有さない酵素阻害剤の開発を試みた。

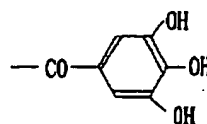
## 10 【0003】

【課題を解決するための手段】本発明者らは化学合成品でなく、天然物の中から目的とする薬効を有する物質を検索すべく鋭意研究を重ねた結果、茶および茶ポリフェノール類に該物質が含まれていることを見出し、本発明に到達した。すなわち、本発明は茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤を提供するものである。本発明のヒアルロニダーゼ活性阻害剤の主成分である茶ポリフェノール類は、下記の一般式Iで表される茶カテキン類と一般式IIで表される茶テアフラビン類である。

## 【0004】

## 【化1】

## 【化3】

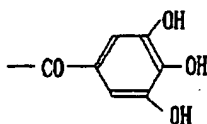


【0010】のもの)

(-)エピガロカテキンガレート(一般式I中、R<sub>1</sub> = OH, R<sub>2</sub> =

## 【0011】

## 【化4】

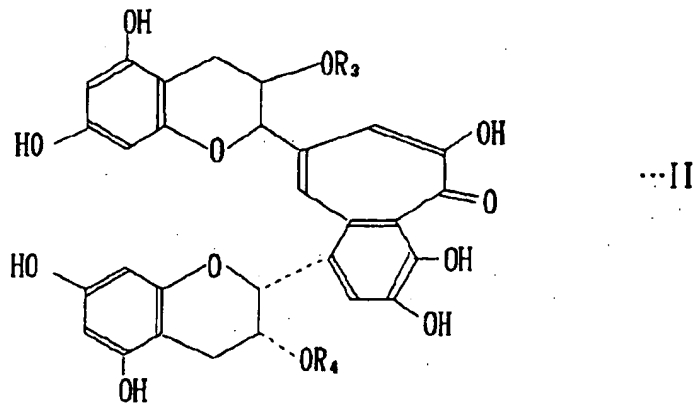


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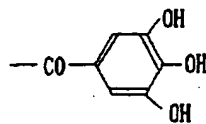
一般式II

## 【0013】

## 【化5】



【0014】(式中、R<sub>3</sub> 及びR<sub>4</sub> はHまたは  
【0015】  
【化6】



【0016】を示し、R<sub>3</sub> 及びR<sub>4</sub> は同じであっても異な  
なっているもの。) )

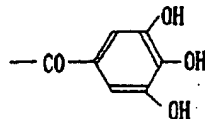
【0017】次に、上記の一般式IIで表される茶テア  
フラビン類を具体的に示すと、以下のものがある。

遊離型テアフラビン (一般式II中、R<sub>3</sub> = H, R<sub>4</sub> = H  
のもの)

テアフラビンモノガラートA (一般式II中、R<sub>3</sub> =

【0018】

【化7】

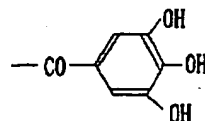


【0019】、R<sub>4</sub> = Hのもの)

テアフラビンモノガラートB (一般式II中、R<sub>3</sub> = H,  
R<sub>4</sub> =

【0020】

【化8】

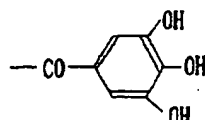


【0021】のもの)

テアフラビンジガラート (一般式II中、R<sub>3</sub> , R<sub>4</sub> =

【0022】

【化9】



【0023】のもの)

上記茶ポリフェノール類は茶葉を原料として製造するこ

とができ、その製法は特開昭59-219384号公  
報、同60-13780号公報、同61-130285  
号公報などに記載されている。

【0024】本発明のヒアルロニダーゼ活性阻害剤は、  
単独で使用する他、適当な賦形剤、例えばゼラチン、ア  
ルギン酸ナトリウムなどと混合したり、水、アルコール  
類などの溶媒、カルボキシメチルセルロースなどの希釈  
剤等と組合わせて用いられる。本発明のヒアルロニダー  
ゼ活性阻害剤の使用量については、薬剤として人体に投  
与する場合、通常は1日量が0.5~10g、好ましくは  
1~3g程度となるように経口的に服用すればよい。剤  
形は任意で散剤、錠剤、カプセル剤などとして用いる。  
また、化粧品などに配合する場合は、最終濃度が1~1  
00ppmとなるように添加すればよい。

【0025】

【実施例】次に、本発明を実施例により詳しく説明す  
る。なお、阻害能の測定は常法に基づき次の方法で行っ  
た。ヒアルロニダーゼ活性阻害剤溶液 (1mM) 50μl  
に酵素 (from Bovine testis, SIGMA 社製) 溶液100μl  
(2,000 unit/ml緩衝液) を加え、37℃で20分間放  
置する。次に、酵素活性化剤 (商品名: compound 48/8  
0, SIGMA 社製) 溶液 (0.1mg/ml緩衝液) 100μl を  
加え、37℃で20分間放置した後、基質であるヒアル  
ロン酸 (from rooster comb, WAKO 社製) 溶液 (0.8 mg  
/ml 緩衝液) 250μl を入れ37℃で40分間放置し  
た。0.4N NaOH 100μl を加えて反応を停止させた  
後、Morgan-Elson法の変法 (Davidson, E. A., Aronson,  
N. N.: J. Biol. Chem. 242, 437(1967)) により生成物  
の定量を行い、ヒアルロニダーゼ活性阻害能を求めた。  
阻害活性は次の式から求められる阻害率で表した。ま  
た、緩衝液には0.1M 酢酸緩衝液 (pH 4.0) を用いた。

【0026】

【数1】阻害率(%)=[(A-B)-(C-D)]/(A-B) × 100

【0027】A:対照溶液の585nmにおける吸光度

B:対照溶液blankの585nmにおける吸光度

C:阻害剤溶液の585nmにおける吸光度

D:阻害剤溶液blankの585nmにおける吸光度

【0028】実施例1

ヒアルロニダーゼ活性阻害剤として第1表に示した各種

茶ポリフェノール類を使用し、上記した方法で各物質の  
ヒアルロニダーゼ活性阻害能を求めた。結果を第1表に  
示す。

\*【0029】

【表1】

\*

第 1 表

阻害剤	阻害率(%)
(+) カテキン	26.8
エピカテキン	22.1
エピガロカテキン	17.6
エピカテキンガレート	55.5
エピガロカテキンガレート	40.3
遊離型テアフラビン	12.2
テアフラビンモノガレートA	85.0
テアフラビンモノガレートB	86.2
テアフラビンジガレート	99.1

【0030】表から明らかなように、カテキン類とテア  
フラビン類の中ではエピカテキンガレート、エピガロカ  
テキンガレート及びテアフラビンモノガレートA、テア  
フラビンモノガレートB、テアフラビンジガレートが強  
いヒアルロニダーゼ活性阻害能を持つことが確認され  
た。

【0031】

【発明の効果】本発明のヒアルロニダーゼ活性阻害剤 ※

※は、日常相当量飲用されている茶から抽出される天然物  
を主成分とするため、薬剤としても人体に対する副作用  
の心配がない。しかも、本発明のヒアルロニダーゼ活性  
阻害剤は低濃度の添加でヒアルロニダーゼの活性化を著  
しく阻害する。したがって、本発明のヒアルロニダーゼ  
活性阻害剤は医薬として各種炎症やアレルギーを軽減す  
るために用いる他、化粧品等に添加してヒアルロン酸の  
分解を防ぐことができる。

フロントページの続き

(51)Int.Cl.<sup>5</sup>

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